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Research Article

CO_2 Supplementation Unlocks Biomass, Lipid, and Carotenoid Potentials in Microalgae *Coelastrella* sp. strain Saripa

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ABSTRACT

Microalgae are a group of photosynthetic microorganisms widely distributed in various habitats, especially in aquatic environment. This study aimed to evaluate the effects of carbon dioxide (CO_2) gas supplementation on the growth and biomass production of the microalgae *Coelastrella* sp. strain Saripa, as well as the lipid and carotenoid contents produced. Additionally, color changes during the growth process were observed as physiological indicators of the microalgae's response to CO_2 treatments and pH changes during growth. The experiment was conducted using a Completely Randomized Design. CO_2 was supplemented at 0, 25, and 50 ml volumes. The results showed that different CO_2 supplementations influenced the pH change, ranging from 6 to 8.4 during cultivation. The 50 ml CO_2 supplementation at pH 6 resulted in the highest biomass production (0.418±0.022 mg/l), lipid production (46%), and total carotenoids (21%). Under this condition, the culture color changed gradually, correlated with the change of chlorophylls and carotenoid contents. Statistical analysis ($\alpha = 0.05$) confirmed that CO_2 supplementations significantly affected biomass, lipid, and carotenoid productions. In conclusion, *Coelastrella* sp. strain Saripa demonstrated potentials in reducing atmospheric CO_2, providing the biomass as lipid and carotenoid bioresources.

Keywords: *Coelastrella* sp., microalgae, CO_2 gas supplementation

Introduction

Microalgae are a group of photosynthetic microorganisms that are found in various habitats, especially aquatic environments. Microalgae play an important role in aquatic ecosystems, including producing oxygen and absorbing CO_2 through photosynthesis. In addition, these photosynthetic microbes possess substantial potential as a food source, animal feed, and raw material for biofuel production.

Coelastrella sp. is a noteworthy microalgae for research due to its high lipid and carotenoid content (Eka, 2021). *Coelastrella* sp. can grow within an optimal temperature ranged from 25°C to 30°C. However, the study of *Coelastrella* sp. is still limited, research is needed to understand its growth characteristics and the factors influencing the production of biomass and bioactive compounds.

CO_2 is a causal factor that can alter microalgae growth. Previous research has shown that increasing CO_2 concentration can enhance the photosynthetic rate and biomass accumulation of microalgae (Yani, 2023). Research on CO_2 supplementation

and microalgae biomass production has emerged as a critical area of inquiry due to its potential to mitigate climate change by reducing greenhouse gas emissions and producing valuable bioproducts. A critical knowledge gap exists in understanding the trade-offs between CO_2 supplementation strategies—such as direct CO_2 bubbling—and their impacts on biomass productivity. This research aims to evaluate the effect of CO_2 supplementation on the production of biomass, lipids, and carotenoids in microalgae *Coelastrella* sp. strain Saripa.

Materials and Methods

Microalgae Cultivation

The strain *Coelastrella* sp. used in this study was collected from Saripa Cave, located in Samangki Village, Simbang District, Maros Regency, South Sulawesi, Indonesia (GPS: 5°02'35''S 119°42'09''E). Artificial freshwater AF-6 medium was used to grow the microalgae, contained $NaNO_3$, NH_4NO_3, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, Fe-citrate, citric acid, KH_2PO_4, K_2HPO_4, PIV metal solution ($FeCl3.6H_2O$, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, $Na_2MoO_4.2H_2O$, and $Na_2EDTA.2H_2O$), and vitamins (biotin, thiamin HCl, B6, and B12). The AF-6 ingredients were dissolved in 1 l freshwater and autoclaved. *Coelastrella* sp. cultures (50 ml) was placed on plastic bioreactor and agitated using an orbital shaker at 135 rpm and 5,000 lux of light intensity. Cultivation was carried out for 14 days after addition of 0 (control), 25, and 50 ml CO_2.

Morphology Observation

The morphological characteristics of the microalgae were examined using the light microscope (Olympus CX43; Tokyo, Japan) under magnifications of 400×.

Research Design

The experiment used a Completely Randomized Design. The data obtained were analyzed using analysis of variance (ANOVA) followed by Duncan's post hoc test.

Analysis of Coelastrella Growth and Biomass Production

The growth was assessed every two days, using the measurement of dry cell weight. The culture samples were collected in volumes of up to 1 ml in a microtube using a 10 ml syringe. The samples were centrifuged at 10,000 rpm and 10°C for 10 min. The pellets were obtained and desiccated in an oven at 60°C for 24 to 48 h. Gravimetric calculations were employed to get growth data over 14-day cultivation period. The growth curve was derived using the graph of growth values recorded every two days. Meanwhile, the specific biomass production (g) was determined utilizing the equation:

$$\text{Specific growth} = \text{Final weight} - \text{Initial weight}$$

Total Lipid Content

Analysis of the lipid content of *Coelastrella* sp. strain Saripa was conducted utilizing the Bligh and Dyer method (1959). Ten milligrams of dried biomass were placed in a test tube and subsequently combined with methanol-chloroform solution (1:1 [v/v]). The sample solutions were vortexed for 30 s, followed by the addition of 1 ml distilled water. The solutions were subsequently centrifuged at 6,000 rpm for a duration of 5 min. The pellet (lipid layer) was transferred to a pre-weighed Petri plate. The procedure was reiterated by including methanol-chloroform-distilled water solution in a ratio of 2:2:1 (v/v) until the solution became transparent. Samples were dehydrated in a vacuum oven and weighed to determine the percentage of total lipid content.

Total Carotenoid Content

One milligram of dry biomass was collected into microtubes, to which 0.5 g of glass beads (0.5 mm and/or 0.2 mm) and 1 ml of cold 80% acetone solvent were added. The entire procedure was conducted in darkness to maintain pigment quality. The solution was vortexed for 20 min until homogeneous, followed by 5 min of centrifugation at 8,000 rpm and 10°C. The

supernatant was transferred to a new test tube covered with aluminum foil. Following several extractions until the biomass was devoid of color, the supernatant containing pigments was ultimately dried in a fume hood or vacuum oven. Two milliliters of acetone were added to the solution containing isolated pigments. Absorbance was quantified at the wavelengths of 662, 645, and 470 nm. Equations for determining chlorophyll-a, chlorophyll-b, and carotenoid concentrations are as follow:

$$\text{Chl-a } (\mu g/ml) = 11.75\ A_{662} - 2.35\ A_{645}$$
$$\text{Chl-b } (\mu g/ml) = 18.61\ A_{645} - 3.96\ A_{662}$$
$$\text{Total car } (\mu g/ml) = 1{,}000\ A_{470} - 2.27\ \text{Chl-a} - 81.4\ \text{Chl-b}$$

Description:
Chl-a = chlorophyll-a content (µg/ml)
Chl-b = chlorophyll-b content (µg/ml)
Total car = carotenoid content (µg/ml)
A_{662} = absorbance at 662 nm
A_{645} = absorbance at 645 nm
A_{470} = absorbance at 470 nm

Color Change Analysis on Culture

This research employed color change analysis of the culture by Red-Green-Blue (RGB) analysis, adhering to the technique established by Patria et al. (2024). The content of chlorophyll and carotenoids associated with the green and red hues in the culture, respectively.

Results and Discussion

Morphological Feature of Coelastrella

Coelastrella sp. strain Saripa observed to grow as single cells, lemon-shaped, and possessing longitudinal ribs. The color on vegetative culture appeared green and transitioned to orange upon the production of carotenoids (Figure 1). The morphological characteristics of the *Coelastrella* examined in this study primarily aligned with the description of *Coelastrella striolata* Chodat, the type species within *Coelastrella* genera. Additionally, this strain shared the same distinctive characteristics as described by Susanti et al. (2024). However, further identification is needed to reveal its species-level identity.

CO_2 Supplementation Impacted Growth and Biomass Production

Microalgae *Coelastrella* sp. strain Saripa was cultured for 8 days with CO_2 gas supplementation at 0, 25, and 50 ml to determine their growth. The adaptation phase begins from day 0 to day 2, followed by the exponential phase until day 8. Meanwhile, the stationary phase was not observable until that period.

Based on the curve shown in Figure 2, it can be seen that CO_2 supplementation enhanced growth of *Coelastrella* sp. strain

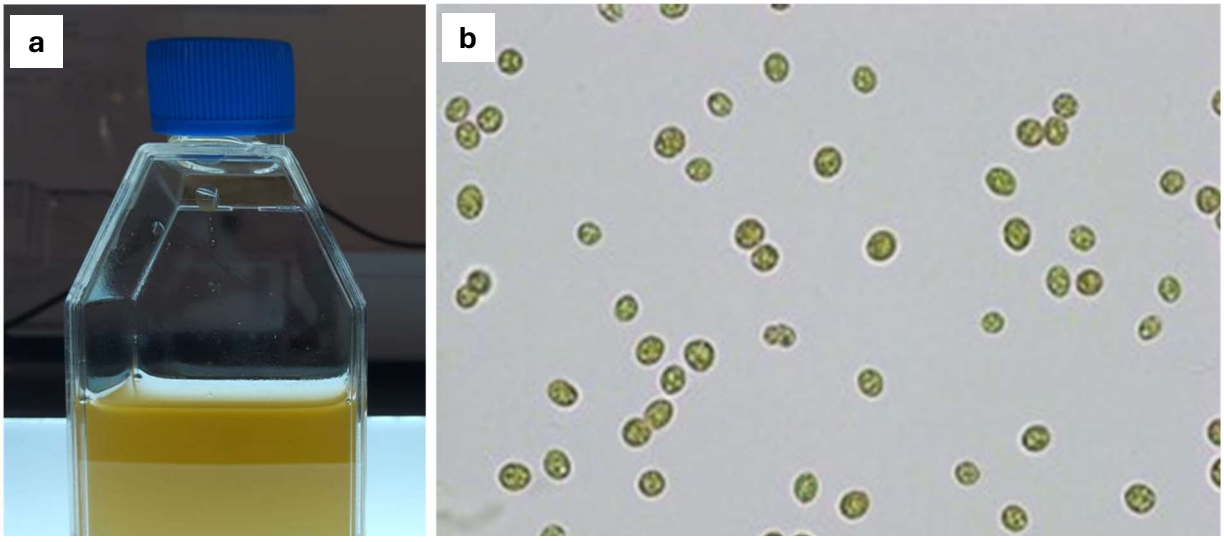

Fig. 1. Morphological feature of *Coelastrella* sp. strain Saripa. (a) the color of culture, (b) cells under magnification 400×.

Saripa by 1.64 to 1.92 times more rapidly than cultures lacking CO_2 supplementation. In the control treatment, where no CO_2 was added, the microalgae growth was slower, with the dry weight that was only increasing slightly from day 0 to day 8, reaching approximately 0.325 g/l at the end of the observation period. Meanwhile, in 50 ml CO_2 supplementation, there was a much higher and consistent increase in dry weight throughout the observation period with the value of 0.625 g/l in day 8. Table 1 illustrated biomass production with the supplementation of CO_2 gas. In addition, cultures without supplemented CO_2 (0 ml) produced the lowest biomass of 0.110±0.0025 g/l, while the addition of 50 ml CO_2 resulted in the highest biomass of 0.418±0.0221 g/l.

The curve in Figure 2 indicates that CO_2 supplementation impacts *Coelastrella* sp. strain Saripa growth. According to Masrun et al. (2022), supplementing CO_2 at the optimal concentration could enhance photosynthetic processes and increase biomass accumulation.

Due to inadequate nutrition, *Coelastrella* sp. strain Saripa grew slower. CO_2 supplementation at 50 ml improves *Coelastrella* sp. strain Saripa growth compared to the control treatment and 25 ml CO_2 supplementation. Previous research supports the importance of optimizing CO_2 concentration for microalgae growth to maximize biomass output (Masrun et al., 2022). This study found that adding 50 ml of CO_2 increased *Coelastrella* sp. strain Saripa biomass production.

The concentration of CO_2 that inhibits microalgae growth varies according to species and environmental conditions. Generally, microalgae can tolerate and even thrive at elevated CO_2 levels, but there is a threshold beyond which growth is inhibited. Molitor et al. (2019) reported that the specific inhibitory concentration can vary, but generally, CO_2 levels above 10% are often detrimental to microalgal growth. Microalgae cells proliferated slower at high CO_2 concentrations due to stress or environmental changes (Masrun et al., 2022).

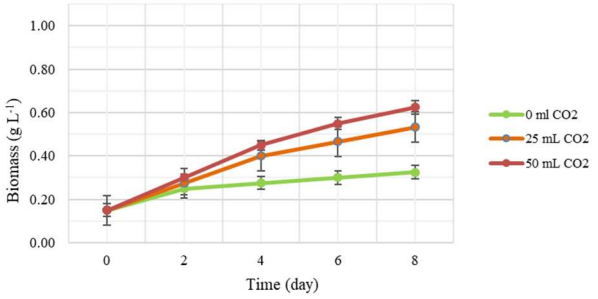

Fig. 2. *Coelastrella* sp. strain Saripa growth upon CO_2 supplementation.

Duncan test results indicate that CO_2 concentration significantly impacts biomass production. Previous study showed that optimizing light and CO_2 variables were crucial for maximizing microalgae biomass productivity (Adriyanti et al., 2021; Hadiyanto & Maulana, 2012). CO_2 supplementation led to changes in photosynthetic activity, the NAD(P)H/NADP+ ratio, and mitochondrial respiration, signifying a modification in energy use inside chloroplasts. The primary mechanisms encompassed state shifts in the photosynthetic system that enhance ATP production, activation of H+-ATPases to regulate cellular pH, and swift cessation of CO_2-concentrating mechanisms. The improved photosynthetic efficiency and optimized metabolic pathways result in the accumulation of valuable biomolecules. Moreover, CO_2 supplementation at specific levels can enhance the efficiency of the carbon-fixing enzyme, Rubisco. Increased CO_2 augment gene expression in the Calvin cycle promotes carbon absorption and stimulates glycolysis, the TCA cycle, and oxidative phosphorylation. This synchronized regulation preserves the carbon/nitrogen equilibrium and supplies ample metabolic energy and carbon frameworks, promoting rapid growth (Peng et al., 2016).

Lipid Accumulation upon CO_2 Supplementation

Based on the research results (Table 1), the lipid content in the microalgae *Coelastrella* sp. strain Saripa is influenced by CO_2 supplementation treatment. In the control treatment, without the addition of CO_2, the lipid content produced was 21%. The addition of 25 ml of CO_2 in treatment increased the lipid content to 29%. Meanwhile, CO_2 supplementation of 50 ml resulted in the lipid content that reached 46%.

Table 1. Biomass and lipid productions upon CO_2 supplementation on *Coelastrella* sp. strain Saripa.

CO_2 (ml)	Biomassa production (g/l)	Lipid (%; w/w)
0	0.110±0.0025[a]	21±1[a]
25	0.326±0.0240[b]	29±1[b]
50	0.418±0.0221[c]	46±1[c]

[a, b, c] significant differences between treatments.

CO_2 supplementation might upregulate genes involved in the Calvin cycle and glycolysis, altering the flow of carbon into lipid biosynthesis pathways (Chu et al., 2019; Sun et al., 2016). In addition, CO_2 supplementation leads to the upregulation of genes involved in triacylglycerol biosynthesis, even when *de novo* fatty acid synthesis genes are downregulated, suggesting a shift in carbon flow towards lipid storage (Sun et al., 2016).

The increase of lipid production obtained in this study underscored the significance of optimizing CO_2 concentration in microalgae cultivation for lipid production, particularly for biodiesel feedstock applications. Optimal concentrations of CO_2 gas and the regulation of additional environmental parameters could markedly enhance lipid productivity and quality in the microalgae *Coelastrella* sp. (Praharyawan, 2021). The Duncan's advanced test indicated that CO_2 concentration treatment significantly affects lipid content (% w/w). Thus, the results showed that CO_2 concentration influence lipid accumulation in microalgae.

Photosynthetic-Associated Pigments in CO_2 Supplementation

Table 2 shows the chlorophyll and carotenoid content of microalgae *Coelastrella* sp. strain Saripa with varying CO_2 gas supplementation. In general, the supplementation of CO_2 consistently elevated the levels of both chlorophyll variants. Similarly, the carotenoid concentration in the microalgae *Coelastrella* sp. strain Saripa exhibited notable increase with the increase of CO_2 supplementation (Table 2). In the control treatment devoid of CO_2 supplementation, the carotenoid concentration was measured at 4 g/l, signifying that in the absence of additional CO_2, carotenoid synthesis is comparatively minimal.

Microalgae contains chlorophyll-a, chlorophyll-b, and carotenoids, which are essential for photosynthetic processes and oxidative stress prevention. Carotenoids protect microalgal cells from free radical damage and maintain photosynthetic stability, especially in unfavorable environmental conditions, as accessory pigments that act as photosynthetic antennas and have strong antioxidant properties. In other words, carotenoid

Table 2. Concentrations of photosynthetic pigments upon CO_2 supplementation on *Coelastrella* sp. strain Saripa.

CO_2 (ml)	Chlorophyll-a (g/l)	Chlorophyll-b (g/l)	Total carotenoids (g/l)
0	0.63±0.024[a]	0.02±0.09[a]	4±0.102[a]
25	0.64±0.026[a]	0.06±0.026[b]	12±0.208[b]
50	0.67±0.01[b]	0.07±0.016[b]	21±0.410[c]

[a, b, c] significant differences between treatments.

pigments protect microalgal cells from oxidative stress that damages cell structure and function during photosynthesis (Aditi et al., 2025; Ren et al., 2021). Previous study suggested that optimal CO_2 concentrations can boost microalgae's bioactive chemical synthesis, particularly carotenoids, as CO_2 is the main carbon source in photosynthesis and pigment biosynthesis (Kandasamy et al., 2021; Yani et al., 2011). This study showed that supplementation of 50 ml of CO_2 leads in the maximum carotenoid content, with a value of 21%. Antioxidant property of carotenoids shields microalgae cells from oxidative stress (Kusnanda et al., 2021). Regulating CO_2 content is crucial for boosting bioactive chemical production in microalgae cultivation (Agustina et al., 2019).

The results showed that CO_2 supplementation boosted the synthesis of chlorophyll-a and chlorophyll-b, indicated by changing in colors. The color changes in the culture were likely due to photosynthetic activity and pigment composition changes within the cells. The strong green color that suggested increased chlorophyll levels, the primary pigment in photosynthesis. Chlorophyll content may be linked to the availability of inorganic carbon as the principal substrate in photosynthesis, which speeds up pigment synthesis (Mulyanto, 2010; Rusdiani et al., 2016). Previous research indicated that high CO_2 levels affecting microalgae development and pigment synthesis (Agustina et al., 2019). Increased CO_2 levels could also activate enzymes involved in lipid and pigment production, leading to color changes in the culture. Research by Adriyanti et al. (2021) indicated that adding CO_2, an essential raw element in photosynthetic processes, could boost microalgae biomass and chlorophyll production.

This study found that 25 ml of CO_2 gas supplementation effectively increases chlorophyll levels, green color, reduces blue pigments, and boosts carotenoid content in *Coelastrella* sp. strain Saripa during the 14-day cultivation period. This condition might be ideal for *Coelastrella* sp. strain Saripa growth, photosynthetic activity, and carotenoid production. Appropriate CO_2 concentrations elevate biomass and chlorophyll production, but high concentrations of CO_2 may cause stress and hinder microalgae growth (Adriyanti et al., 2021).

Correlation among Color Changes, Chlorophyll, and Carotenoid

Figure 3 shows the color changes from green to red in the culture during CO_2 supplementation at the end of day 14. There was an increase in green and red color saturation as CO_2 supplementation increases. Red saturation was higher compared to green at 50 ml CO_2 supplementation, causing the culture to appear orange, which indicates carotenoid production as shown in Table 2.

The correlation coefficient value between the percentage of green color and

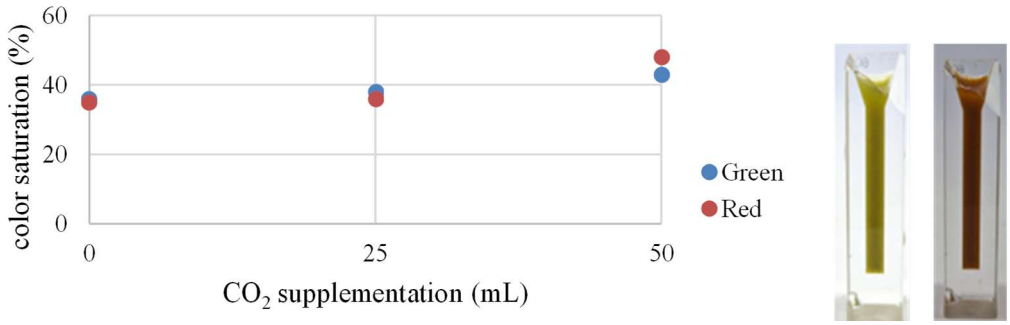

Fig. 3. Green and red color changes upon CO_2 supplementation at the end of day 14 cultivation period.

chlorophyll content is 0.915, while the correlation coefficient between the proportion of red color and carotenoid concentration is 0.945 (Table 3). Chlorophyll and carotenoid pigments in *Coelastrella* sp. strain Saripa cells strongly affected its green and red color variations. The dominating green color in the culture suggested a high abundance of chlorophyll, notably chlorophyll-a (Dharmadewi, 2020). The red color in the culture mainly represented carotenoid pigments, which protect cells from oxidative stress and absorb light (Dharmadewi, 2020).

Table 3. Correlation among colors, chlorophyll, and carotenoid.

	Chlorophyll	Carotenoid
Green	0.915	-
Red	-	0.945

RGB analysis utilized in this study is scientifically significant for elucidating color variations in a culture, as it offers an objective, quantifiable, and reproducible approach to color measurement utilizing readily accessible digital image technology. This approach transforms a subjective visual assessment into definitive numerical data, suitable for thorough scientific investigation. Variations in RGB values can be closely associated with particular chemical or biological processes taking place in the culture. Transitions in the coloration of culture from green to yellow or orange signify alterations in the concentration of pigments such as chlorophylls or carotenoids, which subsequently reflect the physiological condition or stress levels of the organisms.

The correlation study enhances the comprehension of color change in the microalgae cultivation. Therefore, *Coelastrell*a sp. strain Saripa might serve as a reliable indication for assessing the concentrations of primary pigments, specifically chlorophyll and carotenoids. The correlation between green color and chlorophyll (r = 0.915) indicates that the green hue observed in the culture directly corresponds to the chlorophyll content, the primary pigment in photosynthesis that significantly influences microalgae growth (Alfisyahrin et al., 2025; Turnip, 2019). The connection between red color and carotenoid concentration (r = 0.945) suggests that red color intensity serves as a reliable indication of carotenoid levels. Carotenoids serve as protective pigments against oxidative stress and facilitate light absorption in microalgae (Alfisyahrin et al., 2025).

The findings of this study demonstrate that both visual and digital color observation are effective and efficient techniques for monitoring variations in pigment content in microalgae during the cultivation phase. Consequently, the alteration in color of microalgae cultures may functions as a physiological and biochemical marker in microalgae research and biotechnological applications.

Influence of CO_2 Supplementation on the pH Dynamic

The pH dynamic of the microalgae *Coelastrella* sp. strain Saripa during CO_2 supplementation are illustrated in Figure 4. Cultivation of *Coelastrella* sp. strain Saripa without CO_2 gas supplementation (0 ml) resulted in the increase of pH levels from 6 to 9.3 across the cultivation period. The pH of the culture with 25 ml CO_2 supplementation increased during the cultivation period, from 6 to 8.4. Meanwhile, the pH value decreased from day 6 to day 8 with a 50 ml CO_2 gas supplementation.

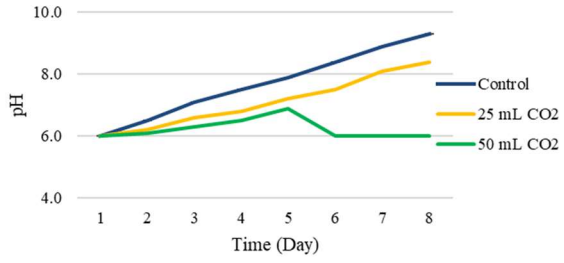

Fig. 4. The effects of CO_2 supplementation on the dynamics of pH during *Coelastrella* sp. strain Saripa cultivation.

CO_2 supplementation plays a critical role in shaping the pH dynamics of microalgae cultures by increasing carbonic acid (H_2CO_3) formation, thereby lowering the pH. Since pH strongly influences cellular metabolism and photosynthetic efficiency, maintaining an optimal range of 6–7 is essential for effective biomass production (Sun et al., 2025). Excessive CO_2 levels elevate dissolved inorganic carbon species (H_2CO_3, HCO_3^-, and CO_3^{2-}), leading to acidification that can impair photosynthetic activity and CO_2 fixation capacity. Such conditions may also suppress extracellular carbonic enzyme activity, further limiting CO_2 uptake and growth (Doney et al., 2009). Moreover, pH fluctuations can alter the ionization state of amino acids, disrupt enzyme conformation, and reduce metabolic efficiency (Satoh et al., 2001). Deviations from the optimal pH range, therefore, disrupt the balance among dissolved inorganic carbon species and impede key physiological processes, including photosynthesis, nutrient assimilation, and cell proliferation (Römheld & Ceci, 2023). The findings of this study confirmed the influence of CO_2 levels on the microalgal pH. Thus, regulating CO_2 level to optimal pH for microalgal growth is expected to improve productivity and metabolic efficiency.

Future Prospects

This study provides a fundamental comprehension of the impact of CO_2 supplementation on the growth, lipid accumulation, and carotenoid production of *Coelastrella* sp. strain Saripa, Future research should clarify the species functional characteristics and identify its bioactive components for possible applications in medicinal, nutraceutical, and cosmetic fields. Moreover, the systematic optimization of culture parameters, such as light intensity, nutrient composition, temperature, and CO_2 concentration, will be essential to enhance the synthesis of high-value metabolites. To facilitate industrial implementation, subsequent research should address large-scale cultivation issues through improved bioreactor design, adopting continuous cultivation methods, and conducting economic feasibility assessments. Comprehensive life cycle assessments will be essential to determine the wider environmental impacts of extensive *Coelastrella* cultivation.

Conclusion

This study demonstrates that *Coelastrella* sp. strain Saripa possesses strong potential as a sustainable bioresource for atmospheric CO_2 mitigation and the production of lipids and carotenoids. The results reveal that varying CO_2 concentrations significantly influence cellular growth, biomass accumulation, and metabolite synthesis, with CO_2 supplementation markedly enhancing lipid and carotenoid yields. Observable color changes during cultivation reflect distinct physiological responses to CO_2 enrichment, while corresponding pH shifts (6.0–8.4) indicate dynamic metabolic activity under different gas conditions. The novelty of this work lies in establishing *Coelastrella* sp. strain Saripa as an effective model for optimizing CO_2 bio-fixation and high-value metabolite production under controlled supplementation regimes. These findings advance understanding of CO_2-driven metabolic regulation in microalgae and provide a foundation for developing scalable biotechnological processes. Future research should focus on large-scale cultivation, process optimization across broader CO_2 ranges, and techno-economic assessments to enhance the feasibility of industrial biomass, lipid, and carotenoid production for bioenergy and bioproduct applications.

Conflict of Interest The authors declare that they have no conflict of interest.

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